

Applicant : Shuping Tong et al.
Serial No. : 09/818,066
Filed : March 27, 2001
Page : 2 of 7

Attorney's Docket No.: 00786-287004 / MGH-0960.3
Tong - Divisional

In the Specification:

Please insert the following paragraphs in the specification at page 8, immediately

following line 12:

ok 3/27
ent 1
3/27
7-8-03

The invention also includes fragments of hepadnavirus pre-S proteins that are capable of binding to receptor p120 and/or p170. In one aspect, the invention provides a polypeptide consisting of (a) a first amino acid sequence that is identical to (1) amino acids 1-104 of a naturally occurring hepadnavirus pre-S protein; or (2) a fragment of amino acids 1-104 of the pre-S protein, provided that the fragment includes at least amino acids 80-102 of the pre-S protein; and (b) one or more amino acid sequences that are not identical to any part of the pre-S protein. The first amino acid sequence can be, for example, amino acids 1-104, 25-104, 42-102, or 59-104 of SEQ ID NO:34, or amino acids 1-102, 25-102, or 59-102 of SEQ ID NO:34. Alternatively, the first amino acid sequence can be amino acids 80-102 or 80-104 of SEQ ID NO:34. In certain embodiments, the polypeptide can include the amino acid sequence of a glutathione S-transferase.

In another aspect, the invention provides a polypeptide consisting of (a) a first amino acid sequence that is identical to (1) amino acids 25-161 of a naturally occurring hepadnavirus pre-S protein; or (2) a fragment of amino acids 25-161 of the pre-S protein, provided that the fragment includes at least amino acids 98-161 of the pre-S protein; and (b) one or more amino acid sequences that are not identical to any part of the pre-S protein. The first amino acid sequence can be, for example, amino acids 87-161, 26-161, 59-161, 71-161, or 80-161 of SEQ ID NO:34. Alternatively, the first amino acid sequence can be amino acids 92-161 or 98-161 of SEQ ID NO:34. In certain embodiments, the polypeptide includes the amino acid sequence of a glutathione S-transferase.

Amend the paragraph on page 19, lines 1 to 13, as follows:

ok 3/27
ent 1
3/27
7-8-03

Detection of pre-S binding proteins in labeled lysates. To reduce the levels of cellular proteins which bind to the GST component of the pre-S fusion protein or directly to the sepharose beads, labeled lysates were first preincubated twice at 4°C with a mixture of empty sepharose beads and GST-bound beads. The beads were washed extensively and used as a negative control in 8% SDS-PAGE. The precleared lysates were then incubated at 4°C for 6 to

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16 hr with the specific GST-pre-S fusion protein. After extensive washing of the beads four times with lysis buffer, bound proteins were eluted from beads by heating to 95°C for 5 min, and separated on 8% SDS-PAGE under denaturing conditions. Proteins retained in the second preclearing reaction were run in parallel. The gel was fixed with 10% acetic acid, treated with AMPLIFY™ fluorographic reagent solution (Amersham), dried, and exposed. For experiments performed with the ¹²⁵I labeled proteins, the treatment step with AMPLIFY™ fluorographic reagent solution was omitted. Comparison of protein bands binding only to the pre-S fusion indicated the specificity of the interaction.
